(51) International Patent Classification 6 : **C11B 3/00, 3/16, C07C 67/48**	**A1**	(11) International Publication Number: **WO 98/32819**
		(43) International Publication Date: 30 July 1998 (30.07.98)

(54) Title: PURIFYING POLYUNSATURATED FATTY ACID GLYCERIDES

(57) Abstract

A composition containing polyunsaturated fatty acid glycerides is purified by means of supercritical fluid fractionation in one or more countercurrent columns operated either with internal reflux achieved by a temperature gradient along the column or with an external reflux achieved through external regulation of the pressure, and using as the solvent a mixture of supercritical CO_2 and a polar co–solvent. Oligomeric and malodorous impurities in particular can be successfully removed by this process, which also can, if desired, be followed, or preceded, by another supercritical fluid fractionation with supercritical CO_2 under conditions adjusted so as to favour fractionation of the glyceride components.

PURIFYING POLYUNSATURATED FATTY ACID GLYCERIDES

This invention relates to a process for treating compositions containing polyunsaturated fatty acid glycerides in order to recover a purified polyunsaturated fatty acid glyceride product therefrom.

5   The fractionation of fatty acids and their derivatives has been widely investigated in recent years. The reason for this interest lies in the recognition that some fatty acids, especially long chain polyunsaturated fatty acids, are precursors for so-called prostanoid
10   compounds, including prostacyclins and prostaglandins, which play an important role in the regulation of biological functions such as platelet aggregation, inflammation and immunological responses.

In this specification polyunsaturated fatty
15   acids are identified according to the system wherein the omega- or n-number denominates the position of the first double bond when counting from the terminal methyl group, e.g. in an omega-3 or n-3 fatty acid, the first double bond occurs at the third carbon atom from the terminal
20   methyl group of the acid. Further, when a fatty acid is identified, for instance, as C18:3, this refers to a fatty acid having 18 carbon atoms in the chain and three double bonds.

Two commercially important polyunsaturated
25   omega-3 fatty acids, EPA (eicosapentaenoic acid, C20:5) and DHA (docosahexaenoic acid, C22:6) are found in marine oils. The biological properties of these fatty acids have been discussed in many publications and patents, such as for instance GB 2221843 which teaches that
30   concentrated mixtures of EPA and DHA are efficient products for the treatment and prophylaxis of multiple risk factors for cardiovascular diseases.

2

Correspondingly, the polyunsaturated fatty acids of the omega-6 series, such as linolenic acid and arachidonic acid, are also of growing commercial importance. The omega-6 acids are commonly produced from

5   vegetable oils such as evening primrose oil and borage oil and are widely employed for pharmaceutical purposes.

These polyunsaturated fatty acids are found in marine and vegetable oils mainly as triglycerides.

As such triglycerides usually also contain

10   unwanted fatty acids, it is often necessary to cut the triglycerides to obtain the fatty acids either in free acid form or as esters with monofunctional alcohols such as methanol and ethanol in order that a proper separation of the desired fatty acids from the unwanted fatty acids

15   can then be accomplished.

On the other hand, for many commercial purposes it is desired that the polyunsaturated fatty acids should be used in the form of glycerides. Accordingly, it is common practice to reconvert purified fatty acids back to

20   glyceride form by esterification with glycerol, or transesterification with glycerol if the fatty acids are present as monofunctional esters. The resulting glyceride products will be termed "synthetic glycerides" hereinafter, to distinguish them from the "natural

25   glycerides" found in the original marine or vegetable oils, although of course they themselves have originated from natural sources as well.

Polyunsaturated fatty acids are extremely fragile when subjected to heat or light in the presence

30   of oxygen and will readily undergo rapid isomerization, peroxidation and oligomerization reactions. Accordingly, even if great care is taken at all times

during the manufacture of purified fatty acid glycerides
for commercial use, it is almost impossible to avoid
several types of organic impurities being present in
either the natural or the synthetic glyceride products,
5   eg in particular:

        (1)   oligomers of fatty acids, which normally
are absent from natural products but typically present up
to 3-5 weight percent, and sometimes at even higher
levels, in synthetic products (they are formed
10   particularly during the esterification or
transesterification with glycerol),

        (2)   malodorous compounds giving an unpleasant
smell and mainly consisting of aldehydes and other
carbonyl compounds resulting from the breakdown of
15   peroxides, and

        (3)   a wide variety of coloured decomposition
products which have not been fully defined by chemical
analysis but which typically impart a darker colour to
synthetic glycerides than to natural glycerides.

20   Over the past decade a number of scientific
groups have focused on the effect of oligomeric (often
called "polymeric") oxidation products on oil quality and
on health.   See, for example, Nawar et al "Stability of
Fish Oils", N-3 News, 1988, 3:3; Kragballe et al
25   "Polyumaettede fedtsyrepraeparater på det danske marked.
Sammensetning og oksidativ stabilitet", Ugeskr. Laeger,
1990, 152:894-897; and Hanmann "What's in those
capsules", Inform, 1990, 1:117-120.   It is generally
recognized that the presence of such comparatively large
30   concentrations of these impurities is undesirable.

4

For these reasons it would be desirable to
reduce the oligomer (polymer) content of both natural and
synthetic fatty acid triglycerides to less than 1% by
weight, preferably to less than 0.5% by weight.

5   The malodorous fatty aldehyde and other
carbonyl components are responsible for the very
unpleasant odour of many oils - they give the very
typical "fishy" smell to marine oils, for example - and
prevent these oils being directly usable in dietary and
10   cosmetic products and even restrain their pharmaceutical
utility.   For this reason it is conventional to steam
deodorise fatty acid glyceride compositions.
However, this process has to be conducted at relatively
high temperatures and can, by itself, give rise to the
15   further production of oligomeric material (see Hanmann,
supra).   Moreover, it should also be noted that
deodorising does not completely remove all oxidation/
decomposition products.   According to the authoritative
Bailey's Industrial Oil and Fat Products (ed. Y.H. Hui,
20   John Wiley & Sons, Inc., New York, 5 ed. 1996, Vol. 4,
page 51):   "While the deodorizer generally represents
the final step in edible oil processing, and can make a
good product from a less than perfect feedstock, the
deodorizer cannot forgive the sins made in earlier
25   processes.   While the primary products of oxidation are
removed (as indicated by the peroxide value), several
damaged oil may contain secondary products of oxidation
(measured as anisidine value).".

The coloured by-products result in the fatty
30   acid glycerides having an unpleasant appearance.
Moreover, it is unsatisfactory to distribute products
containing contaminants which have not been completely
characterised chemically, particularly in the health or
drug fields.

5

        Metal compounds are often used as catalysts in
the production of synthetic polyunsaturated fatty acid
glycerides, and typically include zinc, silver, mercury
and calcium as their salts or oxides.   Residues from the
5   catalysts can dissolve in the glyceride composition,
typically in amount of 1-10 mg/g.   Metals can act as
unwanted catalysts for oxidation.   Also, a purified
product is not expected to contain residues from
catalysts, and the health effects of any such residues
10 should be documented.

        In view of the state of the art it would be
desirable to be able to provide a simple, effective
process for removing at least some of the impurities
which are commonly found in polyunsaturated fatty acid
15 glycerides.

        In accordance with the present invention there
is provided a process for purifying a composition
containing polyunsaturated fatty acid glycerides,
comprising subjecting said composition to supercritical
20 fluid fractionation in one or more countercurrent columns
operated either with internal reflux achieved by a
temperature gradient along the column or with an external
reflux achieved through external regulation of the
pressure, the solvent for said extraction being a mixture
25 of supercritical CO_2 and a polar co-solvent; and
recovering a purified composition containing said
polyunsaturated fatty acid glycerides.

        As is well known, it is possible to change from
one state of a pure compound (i.e. solid, liquid or
30 gaseous) to another state by changing the temperature
and/or pressure of the compound.   It is also well known
that there exists a value, termed the "critical value" of
temperature and/or pressure beyond which it is impossible

to pass from the liquid state to the gaseous state
without ebullition and in the reverse direction without
condensation in a continuous manner.

It is known that a fluid in supercritical
5   state, i.e. in a state characterized either by a pressure
and a temperature respectively higher than the critical
pressure and temperature in the case of a pure compound,
or by a representative point (pressure, temperature)
located beyond the critical point envelop curve
10 represented on a (pressure, temperature) diagram in the
case of a mixture of components, exhibits a high solvent
power for many substances, much higher than that observed
with the same fluid in a compressed gas state. The same
behaviour is observed with "subcritical" liquids, i.e.
15 liquids in a state characterized either by a pressure
higher than the critical pressure and a temperature lower
than the critical temperature, in the case of a pure
compound, or by a pressure higher than the critical
pressure and a temperature lower than the critical
20 temperature of the components in the case of a mixture of
components (see in the journal "Informations Chimie"
No. 321, October 1991, pages 166-177 of the article of
Michel PERRUT, entitled "Les Fluides Supercritiques,
applications en abondance").

25   The important and controllable variations of
the solvent power of such fluids in a supercritical state
are used in many processes: extraction (solid/fluid),
fractionation (liquid/fluid), analytical and preparative
elution chromatography, and material treatment (ceramics,
30 polymers, etc); chemical or biochemical reactions are
also conducted in such solvents.

One of the principal advantages offered by
processes using fluids at a supercritical pressure

7

consists in the easy separation between solvent (the
fluid) and the extracts and solutes, as has been
described in numerous publications.

It has been known for a long time that it is
5   possible to fractionate vegetable or animal oils on
countercurrent columns using supercritical fluids,
especially carbon dioxide or carbon dioxide mixed with an
organic solvent such as propane, hexane and alcohols.
A recently published book edited by J.W. King and
10 G.R. List "Supercritical Fluid Technology in Oil and
Lipid Chemistry", AOCS Press, Champaign, Illinois, USA,
1996 provides a comprehensive review of the published
work in this area.   Chapter 8, "Supercritical Fluid
Extraction and Fractionation of Fish Oils" by
15 W.B. Nilsson, in particular, gives a useful account of
the state of the art relevant to the present invention.

It is an advantage of the process of the
present invention that it is very flexible.
Thus, according to the operating parameters selected it
20 is possible to remove either oligomeric/polymeric
mixtures, or malodorous components, or both, and also the
process can incorporate a step of fractionating the
glyceride components, as will be described below.
Accordingly, the product obtained from the supercritical
25 fractionation can readily be altered at will by
appropriate selection of the operating conditions.

In one preferred embodiment of the present
invention, a glyceride composition which has been
subjected to purification treatment by the present
30 process is then subjected to further supercritical fluid
fractionation in one or more countercurrent columns
operated either with internal reflux achieved by a
temperature gradient along the column or with an external

reflux achieved through external regulation of the
pressure using as solvent a mixture of supercritical CO_2
and a polar solvent, and the fractionation conditions
being controlled so as to favour fractionation of the
5   glyceride components, whereby there is recovered a
triglyceride-enriched fraction and a fraction enriched in
mono- and diglycerides.

In another embodiment of the present invention,
a glyceride composition which has been subjected to
10   removal of mono- and diglycerides is then subjected to
purification by means of the process of the present
invention in order to remove polymeric material.

Achieving fractionation of the glyceride
components requires a higher selectivity than is needed
15   for removal of oligomers. Accordingly, it is preferred
to use conditions leading to a lower solubility of the
glycerides in the solvent. Thus, it is preferred to
operate the fractionation column at a pressure lower than
that used in the column or columns in which the
20   purification step is conducted (typically from 100-250
bar, preferably 120-180 bar), and at a temperature at
least as high, and preferably greater than in the
purification step, and with a similar or lower co-solvent
concentration in the extraction solvent. Typically, the
25   temperature gradient ranges from 30°-40°C (bottom) to
60°-80°C (top), and the co-solvent, preferably ethanol,
is present at a concentration of 5-20% by weight,
preferably 5-10% by weight.

In yet another preferred embodiment of the
30   present invention, the glyceride composition is subjected
to at least two supercritical fractionations in
accordance with the teachings of this invention, with the
operating conditions in one fractionation being selected

9

to favour removal of oligomeric/polymeric impurities, and
those in a second fractionation being selected so as to
favour removal of malodorous components.

As mentioned above, the solvent used in the
5   supercritical fractionation of this invention is a
mixture of supercritical CO_2 and a polar solvent.
By "polar solvent" is meant any organic solvent which is
more polar than unsubstituted saturated hydrocarbons.
Examples of suitable polar solvents for use herein are
10 lower alcohols, such as C_{1-4} alcohols, and lower ketones
such as C_2-C_4 ketones.   Preferred polar co-solvents for
use herein are ethanol and acetone, with ethanol often
being preferred because of its low toxicity.
The presence of the polar co-solvent significantly
15 increases the solvent power of the supercritical CO_2,
whereby the total amount of solvent that has to be
employed can be reduced.

The relative proportions of supercritical CO_2
to polar co-solvent are not critical, but often the
20 weight ratio of supercritical CO_2 to polar co-solvent
will be from 99:1 to 80:20, and preferably from 95:5 to
90:10.

Another important feature of the supercritical
fractionation process of the present invention, leading
25 to enhanced selectivity, is that it is conducted either
with an internal reflux in the column which is achieved
by providing a temperature gradient therealong, or with
an external reflux which is achieved through external
regulation of the pressure.

30 As the solubility of glycerides decreases with
increasing temperature of the solvent mixture, (for
supercritical fluids, solubility usually increases with

10

increasing density and thus decreases with increasing
temperature) the provision of a temperature gradient
along the column, with the temperature rising up the
column, has the effect that some of the components which
5   are initially solubilized when the glyceride composition
is introduced into the column are carried towards the top
of the column in solution.   However, as the temperature
progressively rises these components then come out of
solution again and through the action of gravity travel
10   down towards the bottom of the column where they are
partly re-solubilized.   Thus, the provision of the
internal temperature gradient in the column leads to a
reflux effect which results in a better separation of
impurities from the glyceride composition.   For instance,
15   as shown in Examples 1 and 2 below the provision of
internal reflux leads to the fraction recovered from the
top of the column being substantially free of the less
soluble oligomeric impurities.

Similarly, the provision of external reflux
20   also leads to higher fractionation selectivity.
External reflux is achieved by reducing somewhat the
pressure of the extract withdrawn from the upper end of
the fractionation column, whereby those components of the
extract which have the lowest solubility in the CO_2
25   solvent are caused to precipitate out.   In order to bring
the precipitated components back to the fractionation
column, preferably being reintroduced to the upper part
of the column, the pressure is returned to a higher
level, for example by means of a high pressure pump.

30   The use of external reflux will often be
preferred over internal reflux for larger diameter
columns, where it can often be difficult to establish the
required temperature gradient along the column.

The present invention may be used to purify a wide range of compositions containing polyunsaturated fatty acid glycerides. It is, however, particularly valuable for the purification of glyceride compositions
5 derived from marine oils, in the preparation of pharmaceutical compositions containing high concentrations of EPA + DHA.

By means of the present invention in preferred cases it is possible to substantially remove more than
10 90% by weight of all the aforementioned organic and metallic impurities from polyunsaturated fatty acid glyceride compositions.

The invention will now be described in more detail with reference to the accompanying drawings, in
15 which:

Fig. 1 is a schematic diagram of a first embodiment of a system for treating a polyunsaturated fatty acid glyceride composition of this invention,

Fig. 2 is a schematic diagram of a second
20 embodiment of a system for treating a polyunsaturated fatty acid glyceride composition of this invention,

Fig. 3 is a schematic diagram of the system employed in Example 1,

Fig. 4 is a schematic diagram of the system
25 employed in Example 2,

Fig. 5 is a schematic diagram of the system employed in Example 3, and

Fig. 6 is a schematic diagram of the system employed in Example 4.

12

Referring first to Fig. 1 of the drawings there
is schematically shown a system embodying two independent
supercritical fluid circuits for treating polyunsaturated
fatty acid glycerides in order to remove oligomer and
5   malodorous impurities.

The glyceride composition ("oil") to be treated
is pumped into countercurrent fractionation column 1
which, as indicated, contains at least four separately
heated sections.   The column 1 is filled with inert
10   packing, e.g. glass, PTFE, polyamide, and operates with a
temperature gradient ranging from 30°-40°C (bottom) to
70°-80°C (top) and a pressure between 150-350 bar,
preferably about 250 bar.

Supercritical fluid consisting of CO_2 + polar
15   co-solvent, preferably ethanol at a concentration of
5-10% by weight, is pressurized by means of a pump 4 and
passed through a heater 5 into the lower end of column 1.
The upwardly flowing supercritical fluid contacts the
falling glyceride composition countercurrently, whereby
20   the lower molecular weight compounds including the
desired glycerides are enriched in the fluid.
Similarly, the higher molecular weight species, including
the unwanted oligomers, concentrate in the liquid phase
leaving the bottom of column 1 via expansion vessel 6.

25   The existence in the column 1 of the
temperature gradient provides an internal reflux which
causes higher molecular weight components of the solute,
especially oligomers, to precipitate and thus increases
the selectivity of fractionation.   A similar effect can
30   be obtained by causing reflux of the extract by external
regulation of the pressure using a pump.

Supercritical fluid leaving the top of the
column 1 is expanded to 100 to 200 bar, preferably near

150 bar, and heated up to 60°-80°C in a vessel 2. Under these conditions, the glyceride fraction is precipitated from the supercritical phase of CO_2 + co-solvent which is recycled via a cooler 3, pump 4 and

5   heater 5 to the column 1.

The glyceride composition from vessel 2 containing up to 20% of ethanol co-solvent, is then fed into the top of a second packed column 6 operated at 120-150 bar and temperature of 50°-70°C, where it is

10   countercurrently contacted with pure supercritical CO_2 which preferentially entrains volatile compounds (odours) as well as co-solvent remaining in the glyceride composition.

Volatile fraction-laden CO_2 is routed via an

15   expansion vessel 7 at a pressure of 40-60 bar where most of the volatile components are precipitated, an adsorption vessel 8 filled with activated charcoal to remove traces of volatiles, a cooler 9, a pump 10, a heater 11 and then, finally, is recycled to the column 6.

20   The purified liquid exiting from the bottom of column 6 is expanded in vessel 12 to 40-60 bar and to 1 bar in vessel 13 and the CO_2 from the first decompression vessel 12 is recirculated to cooler 9.

Compared with a typical procedure of

25   supercritical extraction with co-solvent including combined precipitation of extract + co-solvent, the proposed process offers several important advantages. As the glyceride composition from vessel 2 contains up to 20% ethanol as co-solvent, it is unnecessary to add

30   co-solvent to the CO_2 that is used in column 6. The pure CO_2 in column 6 dissolves the co-solvent from the glyceride composition, and thus increases the solubility

14

of the volatile compounds (odours). As the co-solvent is
removed together with the volatile components, no costly
distillation unit is needed in order to remove the
co-solvent from the product, thus simplifying the
5   process, and reducing the thermal treatment of the
product which is required.

Referring now to Fig. 2, there is shown
schematically a system for purifying a polyunsaturated
fatty acid glyceride composition in accordance with this
10 invention using a single supercritical fluid circuit.

As in the system shown in Fig. 1, the glyceride
composition is initially treated in fractionation column
1 with a countercurrent flow of supercritical fluid,
preferably CO_2 + ethanol, which is then led to an
15 expansion vessel 2 to precipitate out the glyceride
phase.

Thereafter, the supercritical phase of CO_2 +
co-solvent leaving vessel 2 is once more expanded to
100-400 bar, preferably about 120 bar, in a vessel 5 to
20 precipitate ethanol from the supercritical phase of CO_2.
Supercritical CO_2, stripped of co-solvent, is purified in
a charcoal absorption bed in a vessel 6, and then enters
column 3 where, as in column 6 of Fig. 1, it flows
countercurrently to the glyceride fraction withdrawn from
25 expansion vessel 2 and extraction of the malodorous
volatile compounds occurs. The column operates at a
constant temperature of 50°-70°C, preferably about 60°C,
and a pressure of 100-140 bar, preferably about 120 bar.
The stream leaving the top of the column 3 is expanded at
30 40-60 bar into a vessel 4 to precipitate malodorous
volatiles and remaining co-solvent from the CO_2.
The gaseous CO_2 which is liberated in vessel 4 is
condensed in a cooler 7, mixed with co-solvent recycled

from vessel 5 and recirculated by means of a pump 8
through a heater 9 back into the column 1.

The purified glyceride composition is withdrawn
from vessel 10.

5   It will be appreciated that, compared with the
system of Fig. 1, the arrangement of Fig. 2 requires only
one cooling/pumping system, thereby effecting
considerable capital and operating costs. Nonetheless,
the system can, like that of Fig. 1, be used to remove
10   both oligomeric and malodorous impurities from glyceride
compositions.

The invention will now be illustrated by the
Examples which follow:

EXAMPLE 1

15   This example illustrates the separation of
oligomers from synthetic polyunsaturated fatty acid
glycerides using a supercritical fluid composed of CO_2
and acetone co-solvent, without co-solvent regeneration.
A single multistage column was used and extract and
20   co-solvent fluid separation are achieved under
subcritical conditions.

The apparatus employed is shown schematically
in Fig. 3.

A 4.5 m long, 8 mm-internal diameter stainless
25   steel column 1 filled with glass Rashig rings was
employed. The column 1 was arranged in four sections
heated by different baths, resulting in a temperature
gradient from 40°C at the bottom to 78°C at the top of
the column.

16

A fish oil (EPAX-5500), available from PRONOVA and characterized as shown in Table 1 was used.

General		Analysis
Acid value	mg KOH/g	2.2
Iodine value	gl/100 g	322.4
Saponification value	mg KOH/g	169.2
Unsaponification value	%	1.4
Peroxide value	meq/kg	4.4
Cholesterol	mg/g	6.6
Colour Gardner		5.7
Fatty acid composition		
Total ω-3	A%	70.8
EPA	A%	34.0
DHA	A%	23.8
Total EPA and DHA	A%	57.8
Tocopherols		
Mixed nat. tocopherols*	mg/g	3.5
Others		
Refractive index	20°C	1.5020

20 *Added as 1.42 mg/g Coviox T-70 (Henkel) and 3 mg/g
    Covitol F-1000 (Henkel)

2 kg of the oil was mixed with 8 kg acetone of technical grade and fed into the first stage of the column (temperature 40°C). The column was operated on
25 continuous mode at 250 bar using the following loading:

- feed flow rate = 0.38 kg/h
- CO_2 flow rate = 20.3 kg/h
- acetone content in CO_2 = 6.5 wt %

The product from the top of the column,
30 containing purified oil and acetone dissolved in supercritical CO_2 was expanded in vessel 2 into

subcritical conditions (20°C, 50 bar) to precipitate
co-solvent and extract.   After acetone vaporization in
vessel 3, 1.34 kg of oil (extraction yield **67%**) was
recovered.   Both the top and bottom fractions were
5   subjected to the analysis for polymers/glycerides content
by gel permeation chromatography (GPC).   The results are
shown in Table 2:

Table 2 (Area %)

Sample	Oligomers	TG	DG	MG	FFA
Top fraction	-	32.6	52.5	12.9	1.9
Bottom fraction	5.6	72.4	20.4	0.6	1.0

        From the data in Table 2, it can be seen that
the process of oligomer separation is highly effective;
the oil from the top is in practice free of heavy,
15   undesirable oligomeric products.

                        EXAMPLE 2

        This example illustrates the separation of
oligomers from the fish oil EPAX-5500 which was utilized
in Example 1, on a multistage countercurrent column using
20   a supercritical fluid composed of CO_2 and ethanol, with
co-solvent recovery and recycling.

        The equipment employed is shown schematically
in Fig. 4;   it will be seen that it is similar to that
shown in Fig. 1.   The column 1 was equipped with three
25   separators operated at different levels of pressure and
temperature.

18

The system was first filled with CO_2 and then the high pressure pump was operated. The operating conditions were as follows:

	Temp (°C)	Pressure (bar)
Column	40 (bottom)/50 (top)	250
Separators 1 + 2	78	150
Separator 3	40	50

5

- feed flow rate = 0.57 kg/h
- solvent flow rate = 24.6 kg/h

10   1.5 kg of ethanol (fermentation grade) was added to the circulating CO_2. After 2 hours, when the content of ethanol in CO_2 was stabilized at the level of 7 wt %, 3 kg of the fish oil was fed into the second stage of the column. Oil-laden fluid leaving the top of the column was introduced into the separator S1 where the oil fraction was precipitated; the carbon dioxide + ethanol remained in the supercritical state; this stream of CO_2 with entrainer leaving separator S1 was passed through vessel S2 filled with glass packing to remove oil droplets and then recycled to the column.

20   Liquid phase from S1 was expanded in separator S3 to decrease the CO_2 content. The ethanol concentration in the products was as follows:

extract (S3)   :   8.8%
residue (column) :   8.9%

Table 3 (Area %)

Sample	Oligomers	TG	DG	MG	FFA
Extract (83%)	0.15	40.8	46.4	10.6	2.0
Residue (17%)	10.1	69.6	19.9	0.4	-

5   As can be seen from Table 3, fish oil
processing with ethanol as a co-solvent was just as
effective in removing oligomeric impurities as the
process described in Example 1. Furthermore, this
process, with co-solvent recovery and recycling, yields
10   products much less contaminated by ethanol which can, if
desired, be easily stripped by CO_2 in an oil
deodorization step such as is illustrated in Example 4.

                          EXAMPLE 3

        This example illustrates the subsequent
15   treatment of purified triglyceride composition by the
process of the invention to recover a triglyceride-
enriched fraction and a mono- and diglyceride-enriched
fraction.

        The apparatus used is schematically illustrated
20   in Fig. 5.

        A multistage column, similar to that employed
in Example 1, was operated under the following
conditions:

        - temperature gradient 40°C (bottom)/50/65/78°C (top)
25   - pressure = 150 bar
        - solvent/feed ratio = 88 kg/kg
        - feed flow rate = 0.275 kg/h
        - solvent flow rate = 24.2 kg/h

688 g of fish oil, subjected to preliminary removal of oligomers by a process similar to Example 2 was fed to the second stage of the column. Ethanol and CO_2 were pre-mixed in the proportions corresponding to

5   co-solvent concentration of 10 wt %. Of the initial feed, 66% was withdrawn at the bottom of the column and 34%, as a mixture with CO_2 + ethanol, left the top of the column and then passed through two separators (20°C/50 bar) to precipitate a glyceride fraction + ethanol and to

10   liberate gaseous CO_2.

The contents of glycerides in the initial oil and in the two fractions obtained were evaluated by HPLC with a refractive index detector. The results are shown in Table 4.

15   Table 4 (Area %)

Sample	TG	DG	MG
Feed	67	27	6
Top Fraction (34%)	4	63	33
Bottom Fraction (66%)	89	10	1

20   These analytical data show that the experiment achieved good separation of triglycerides from di- and mono-glycerides, the bottom fraction consisting of triglycerides.

This example, as well as Example 2, illustrates

25   the flexibility of supercritical fluid processing of glycerides of fatty acids derived from marine oils when using the same binary solvent, CO_2 + ethanol, and changing only the operation parameters of the column: separation of oligomers as well as glyceride

30   fractionation can be achieved.

21

EXAMPLE 4

This example illustrates the purification of
fish oil on a multistage column under conditions
favourable to remove the malodorous impurities.

5   The equipment used is schematically shown in
Fig. 6.

A multistage column, as in Example 1, was
operated under the following conditions:

- temperature gradient = 50°C (bottom)/55/60/70°C (top)
10 - pressure =   120 bar
- CO_2/feed ratio = 38
- feed flow rate = 0.645 kg/h
- CO_2 flow rate = 24.5 kg.h

1.2 kg of fish oil (EPAX 5500) was fed into the
15 top of the column and contacted countercurrently with
supercritical carbon dioxide flowing from the bottom.
Preferentially entrained volatile compounds were
dissolved in CO_2 and left the top of the column.
This odour fraction-laden CO_2 was first expanded into 3
20 cyclonic separators (80/70/50 bar/20°C) to precipitate
liquid extract and then passed through a two litre vessel
filled with activated charcoal to remove traces of
volatiles from CO_2. Carbon dioxide leaving charcoal bed
was, after liquefaction, recycled to the column.

25   1.16 kg of oil was withdrawn at the bottom
(recovery ~ 97%). The results of GC analysis show
imperceptible concentrations of volatile compounds in the
refined product and the fish odour had totally
disappeared.

EXAMPLE 5

This example illustrates the separation of
metal compounds from a glyceride composition.
The equipment employed was identical to that used in
5   Example 2.

Before extraction, the glyceride had been in
contact with zinc oxide power (catalyst for
esterification). After the esterification reaction was
terminated, some zinc remained in solution. The exact
10 chemical composition of the dissolved zinc was unclear,
but the zinc could possibly have been present as soaps or
possibly as complexes with fatty acid double bonds.
Before the extraction started, undissolved powder was
removed by filtering.

15   The column was operated at 250 bar and at a
temperature gradient from 40-60°C. The feed flow rate
was 0.5 kg/h, and the solvent flow rate 25 kg/h.
The content of ethanol in the solvent was 6.5%.

As in Example 2, the oligomers were
20 concentrated in the residue (18% in the residue and below
detection limit in the extract). At the same time, zinc
was concentrated in the residue:

	Zn (mg/g)
Extract (87%)	0.034
25 Residue (13%)	5.6

These results demonstrate that the present
invention represents an excellent process for the removal
of metal contaminants from glycerides.

23

EXAMPLE 6

This example illustrates the use of external reflux.

The process and apparatus are generally similar
5 to that of Example 2 and Fig. 4 except that the
temperatures in the column jackets are set to be uniform
at 40°C.   A high pressure pump recompresses part of the
liquid collected in vessel 51 (150 bar, 78°C) that is
recycled at the top of the column, at a flowrate of
10 0.1 kg.h^{-1}.

External reflux achieves very similar results
in terms of productivity, yield and purity to those
obtained with internal reflux.

24

CLAIMS:

1.   A process for purifying a composition
containing polyunsaturated fatty acid glycerides,
comprising subjecting said composition to supercritical
fluid fractionation in one or more countercurrent columns
operated either with internal reflux achieved by a
temperature gradient along the column or with an external
reflux achieved through external regulation of the
pressure, the solvent for said extraction being a mixture
of supercritical CO_2 and a polar co-solvent; and
recovering a purified composition containing said
polyunsaturated fatty acid glycerides.

2.   A process according to Claim 1, wherein said
polar co-solvent is ethanol or acetone.

3.   A process according to Claim 1 or Claim 2,
wherein the weight ratio of said supercritical CO_2 to
said polar co-solvent is from 99:1 to 80:20.

4.   A process according to any preceding claim,
which is operated so as to preferentially remove
oligomeric/polymeric impurities from said glyceride
composition.

5.   A process according to any one of Claims 1-3,
which is operated so as to preferentially remove metal
impurities from said glyceride composition.

6.   A process according to any one of Claims 1-3,
which is operated so as to preferentially remove
malodorous components from said glyceride composition.

7.   A process according to any preceding claim,
wherein said purified composition which is recovered is

subjected to a supercritical fluid fractionation in one
or more countercurrent columns operated either with
internal reflux achieved by a temperature gradient along
the column or with an external reflux achieved through
external regulation of the pressure, the solvent for said
extraction being a mixture of supercritical CO_2 and a
polar co-solvent, and the fractionation conditions being
controlled so as to favour fractionation of the glyceride
components, whereby there is recovered a fraction
enriched in triglycerides and a fraction enriched in
mono- and diglycerides.

8.   A process according to any preceding claim,
wherein said glyceride composition is derived from a
marine oil and is subjected to said supercritical fluid
fractionation in at least two countercurrent columns the
operating conditions in one said column being selected so
as to preferentially remove oligomeric/polymeric
impurities and the operating conditions in the another
said column being selected so as to preferentially remove
malodorous components.

9.   A process according to Claim 8, wherein the
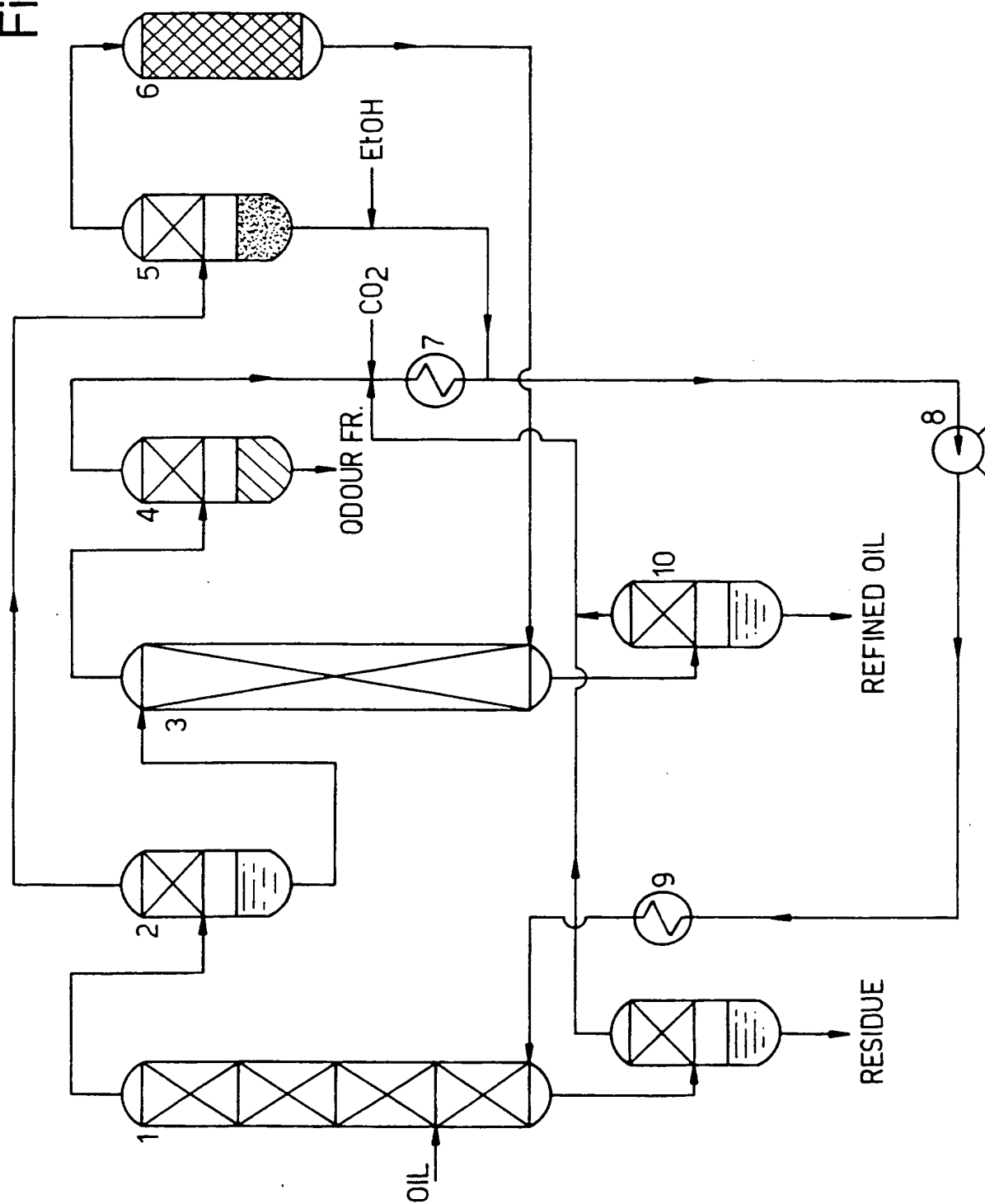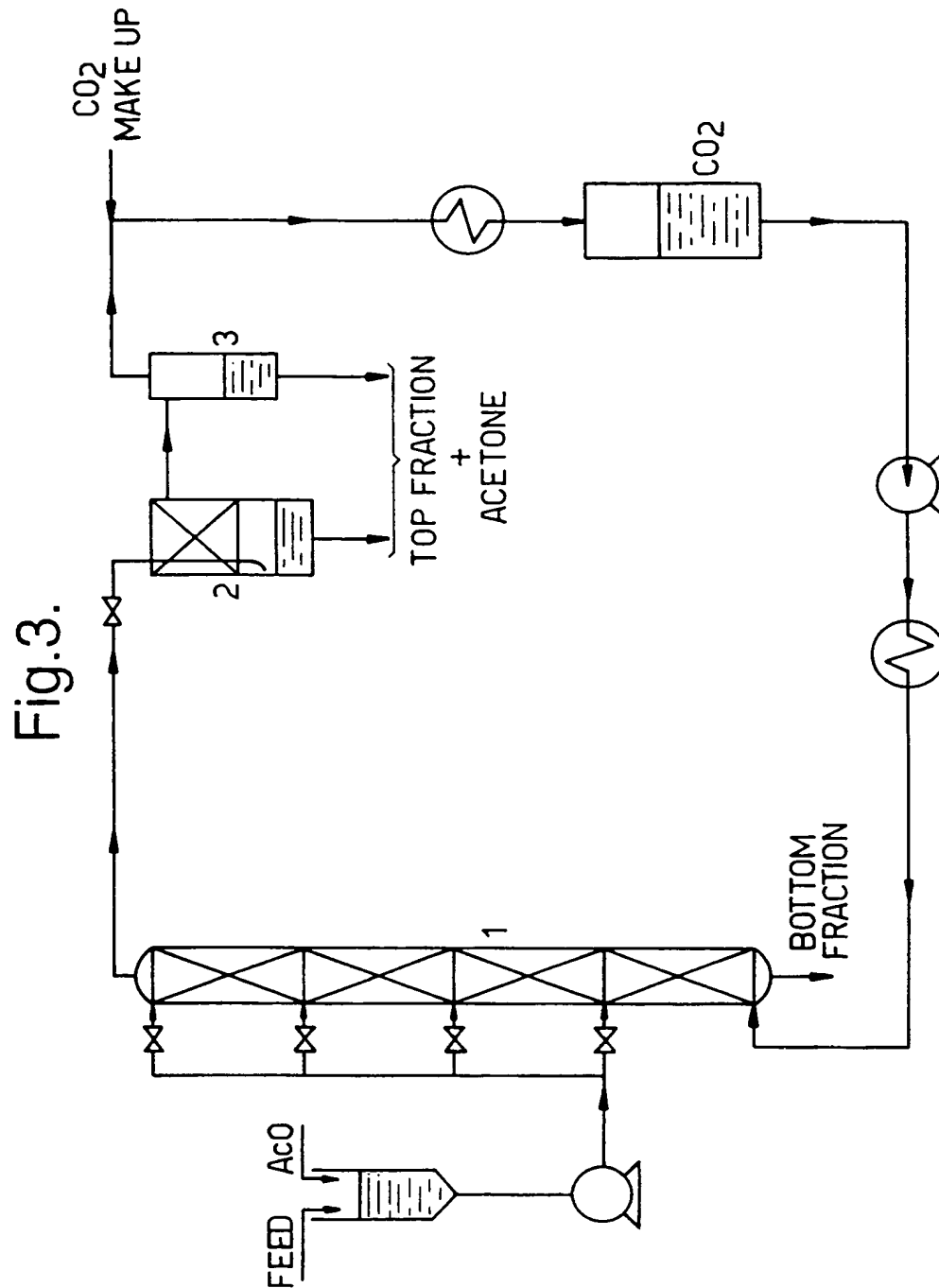solvent in said another column consists essentially of
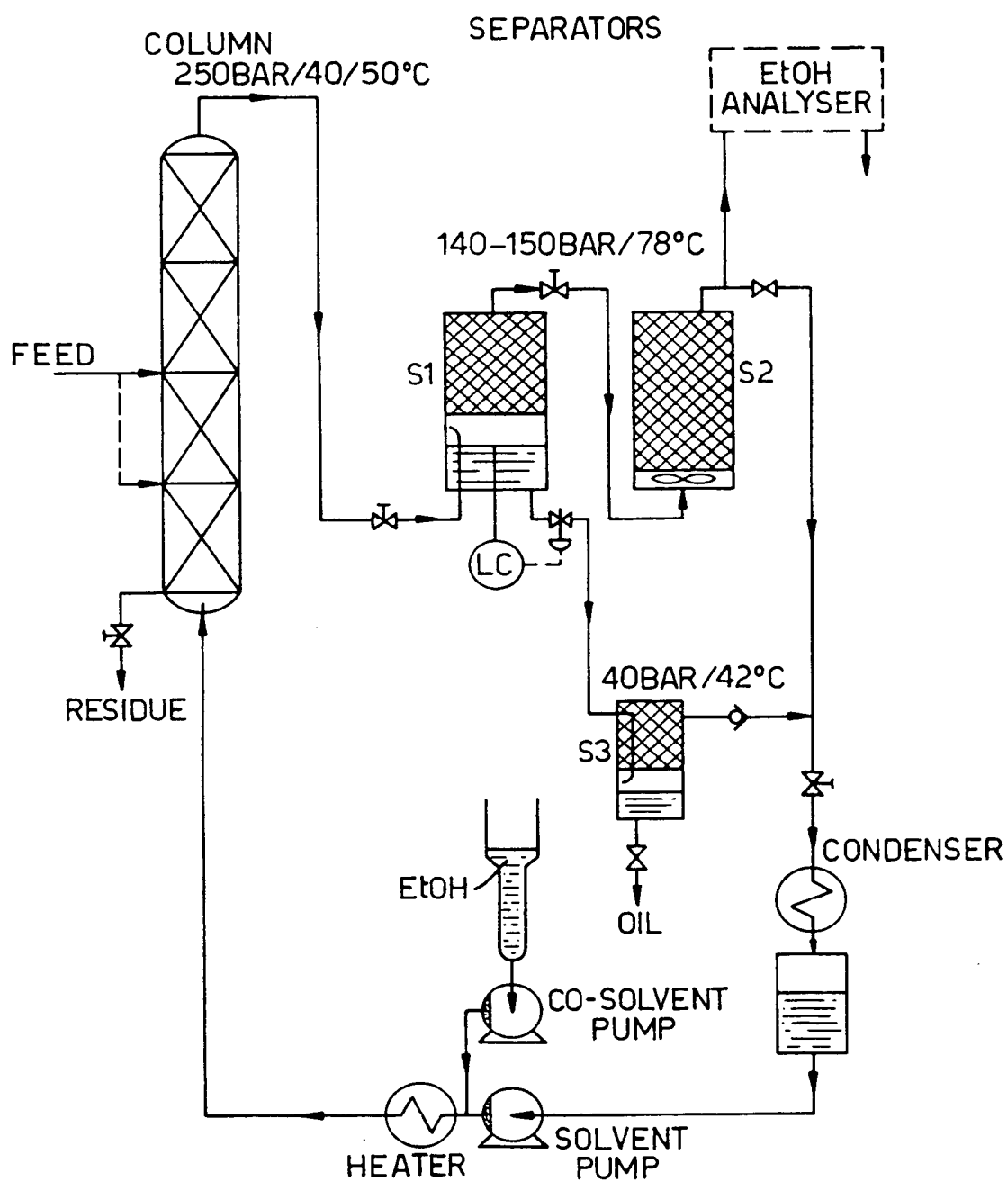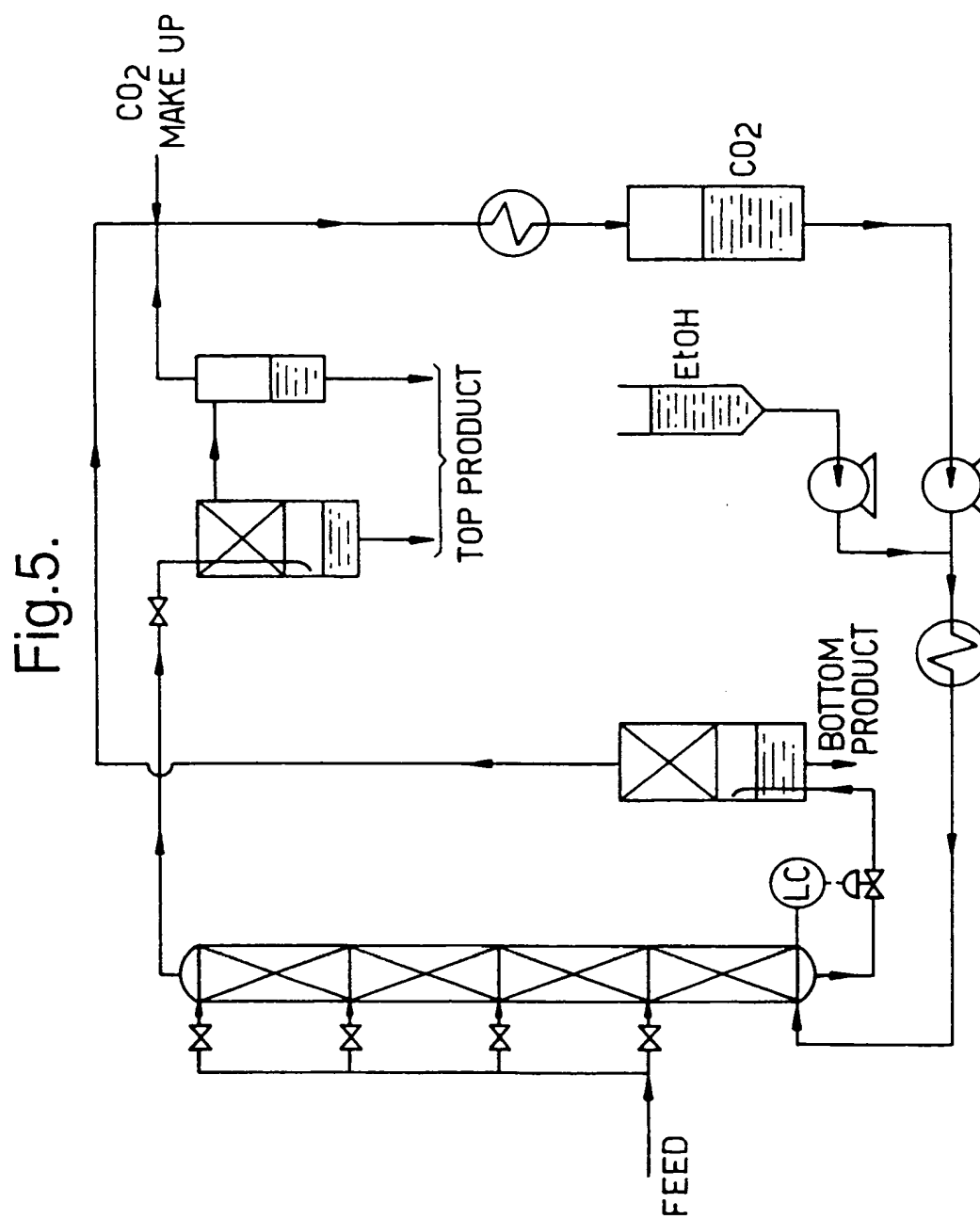supercritical CO_2.
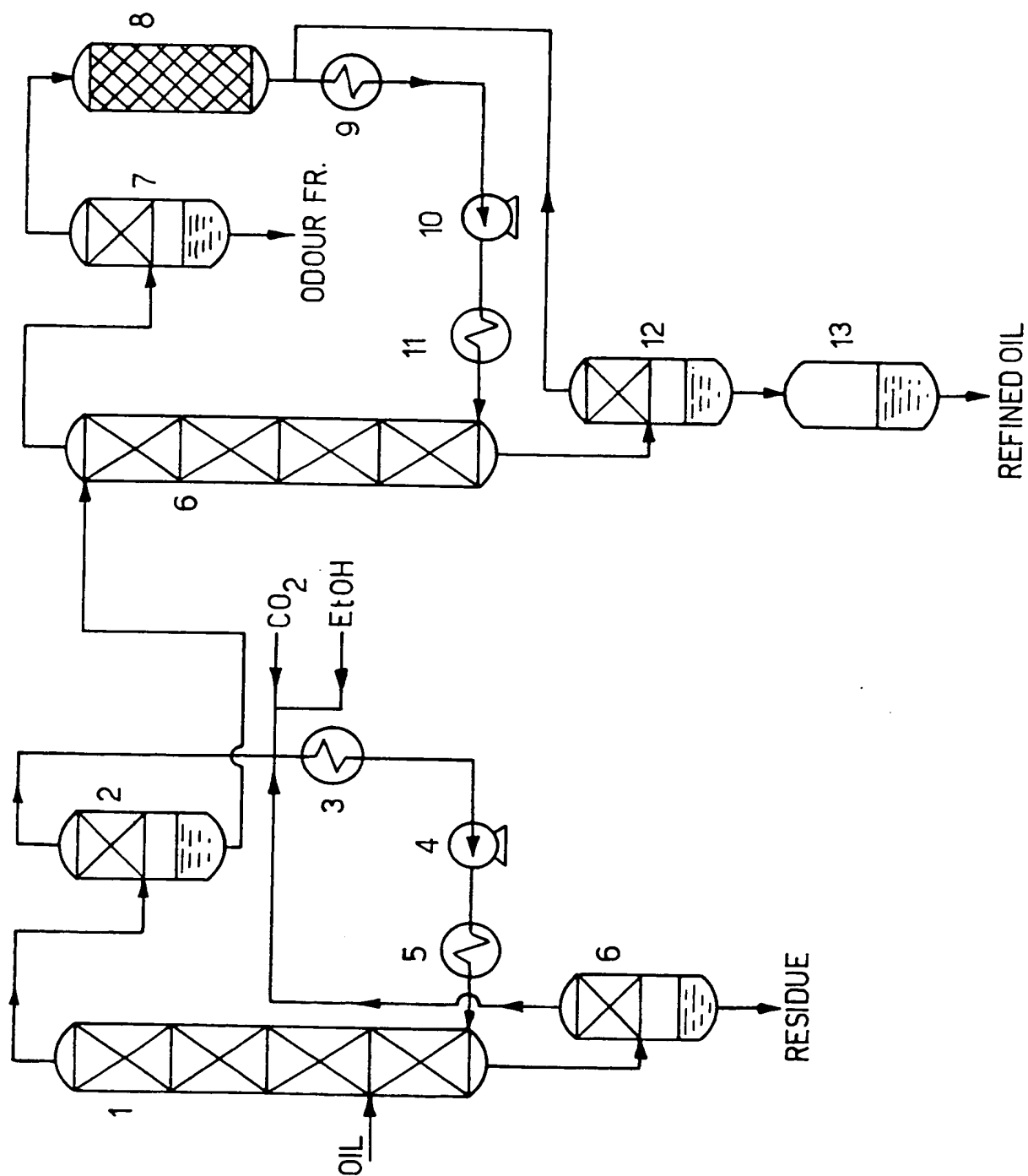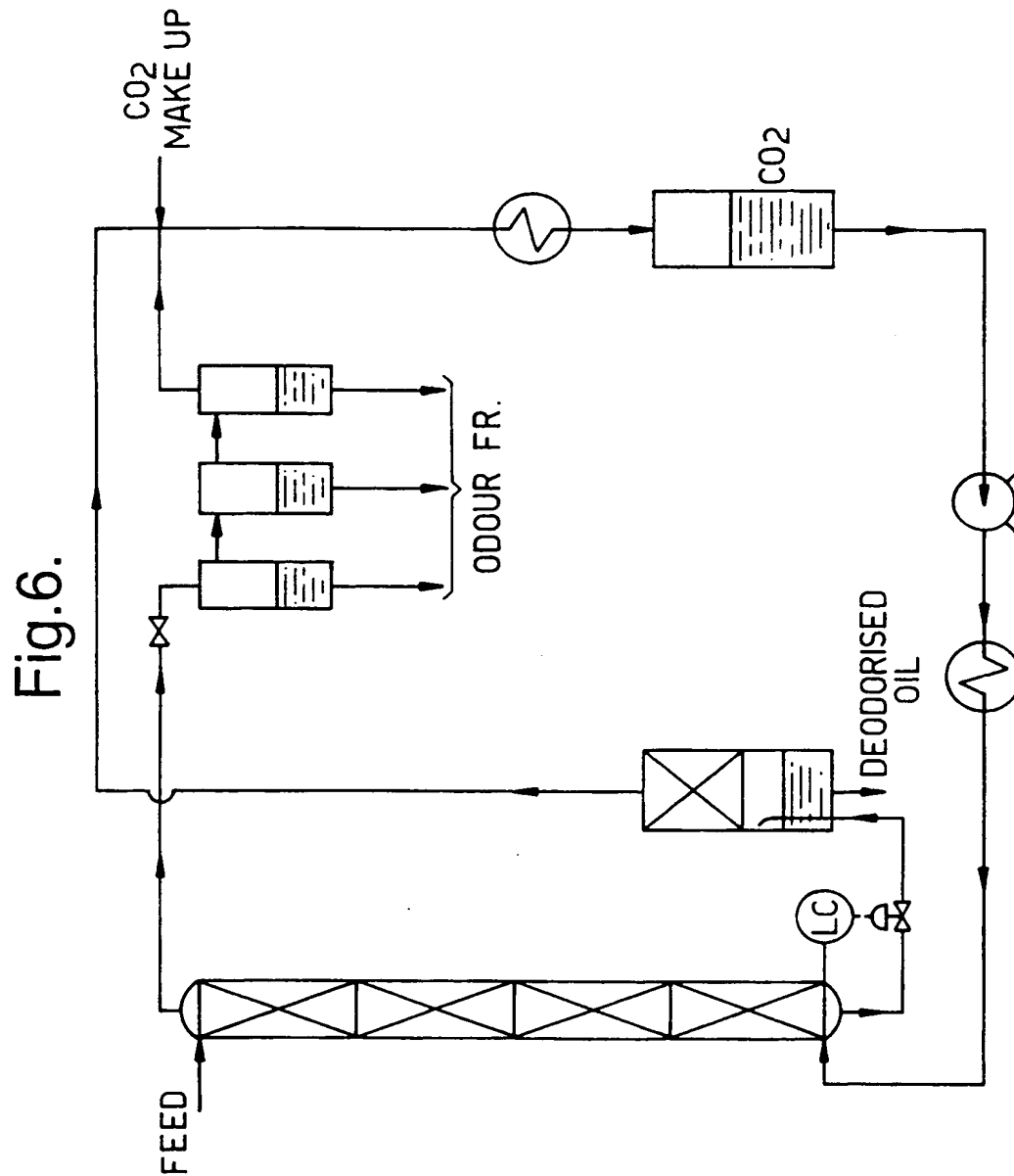
Fig.1.

Fig.2.

Fig.3.

Fig.4.

Fig.5.

Fig.6.

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C11B 3/00, C11B 3/16, C07C 67/48
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C11B, C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	STN International, File CAPLUS, CAPLUS accession no. 122:54551, Kuk, M.S. et al: "Supercritical carbon dioxide extraction of cottonseed with co-solvents", J. Am. Oil Chem. Soc. (1994), 71(12), 1353-6	1-9
	--	
X	Databas Orbit, Japio, Japio accession no. 94-293895, Nakajima, Nobuaki et al: "Method for Separating Triglyceride from Vegetable Oil", JP 06-293895, 941021	1-2
A		3-9
	--	

[X] Further documents are listed in the continuation of Box C.   [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure. use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 May 1998	15 -05- 1998
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Eva Johansson Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1992)

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5013443 A (SAKAE HIGASHIDATE ET AL), 7 May 1991 (07.05.91)	1-2
A		3-9
	--	
X	US 4675132 A (VIRGINIA F. STOUT ET AL), 23 June 1987 (23.06.87)	1-2
A		3-9
	--	
A	STN International, File CAPLUS, CAPLUS accession no. 117:232533, Nilsson, W. B. et al: "Supercritical fluid carbon dioxide fractionation of fish oil esters"; & Adv. Seafood Biochem., Pap. Am. Chem. Soc. Annu. Meet. (1992), Meeting Date 1987, 151-68	1-9
	--	
A	US 4692280 A (JOHN SPINELLI ET AL), 8 Sept 1997 (08.09.97)	1-9
	-- --------	

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5013443 A	07/05/91	DE 69013702 D,T EP 0379963 A,B JP 2194802 A	16/03/95 01/08/90 01/08/90
US 4675132 A	23/06/87	NONE	
US 4692280 A	08/09/97	NONE	